51 Inhaled GSH tolerability in patients with cystic fibrosis (CF)

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Objectives: Oxidative stress biomarkers as reactive oxygen species are induced by the sustained activation of neutrophils and other CF-derived defects in the lung of CF patients. Observed defects include an impaired glutathione (GSH) metabolism. Its supplementation may counterbalance the oxidative stress. A randomized, single blind controlled trial of inhaled GSH versus placebo (NCT01450267) is underway in order to evaluate the effect of GSH in cohort of CF patients. We report preliminary data on tolerability to GSH in a pediatric subset of enrolled patients.
Methods: 48 CF patients (F 23, age M±DS 13.53 yrs), in regular follow up at the Regional Pediatric CF Center of Naples, were enrolled for RCT. The main inclusion criteria were: CF diagnosis by sweat test and/or two CF causing mutations, age of patients >6 yrs, FEV1% >40% of the predicted value, negative culture for *Burkholderia cepacia*. Spirometry was performed before and 10 and 60 minutes after GSH inhalation test (10 mg/kg, maximum dosage 600 mg/dose) in order to assess tolerability.
Conclusions: No patients showed a decrease in FEV1% >15% after GSH inhalation as defined in the study design. A statistically significant increase was observed for FEF25–75% after 10 and 60 minutes from inhalation (FEF_{25-75} M±DS: T_0 71.64±33.35 VS T_{10} 76.37±36.73; $p < 0.02$ and T_0 71.64±33.35 VS T_{60}: 80.26±35.25; $p < 0.0001$) and for FEV1% after 60 minutes from inhalation (FEV_1 M±DS: T_0 97.90±21.03 VS T_{60} 100.01±19.42; $p < 0.01$). No side effects were reported. On the basis of these preliminary results we are currently evaluating the efficacy of inhaled GSH on pulmonary function and inflammatory markers within a 12 months therapy.

52 Nebulized hyaluronan ameliorates lung inflammation in cystic fibrosis (CF) mice

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Objectives: Chronic lung inflammation and bacterial infections cause much of the morbidity and mortality in patients with CF. Previous studies have shown that hyaluronan (HA) may exert a protective effect against injury in experimental models of chronic respiratory diseases. Our objective was to examine if exogenous administration of nebulized HA might interfere with lung inflammation in vivo in mouse models of CF.
Methods: F508del homozygous mice (*CftrF508del*) and transgenic mice overexpressing the ENaC channel β-subunit (Scnn1b-Tg) were treated with nebulized HA (0.5 mg per animal in saline solution for 30 minutes once daily for 7 days). TNFα expression, MIP2 levels, MPO activity and macrophage infiltration were assessed on lung tissues. CF cell lines were cultured with HA (24 h, 100 μg/ml) and Reactive Oxygen Species (ROS), Tissue Transglutaminase (TG2) SUMOylation, PPARγ and phospho-p42/p44 levels were measured by dichlorodihydrofluorescein assay, or FRET microscopy or immunoblots.
Conclusions: Nebulized HA reduced TNFα mRNA levels (52±30%, and 64±32%, $p < 0.005$), MIP2 (56±9% and 79±7%, $p < 0.05$), MPO protein levels (from 1780.47±973.18 to 548.84±386.64 and from 2325.25±840.83 to 1003.12±722.13 nmol/min/ml, $p < 0.05$), CD68+ cells counts in lung tissues (from 102.4±27.1 to 36.6±19.2 and from 62.4±28.2 to 25.8±13.2 per mm^2 of tissue, $p < 0.005$) in both *CftrF508del* and Scnn1b-Tg mice, respectively, as compared to saline-treated mice. HA reduced ROS, TG2 SUMOylation, TG2 enzyme activity, phospho-p42–44 and increased PPARγ protein in both IB3–1 and CFBE41o– cell lines ($p < 0.05$). Inhaled HA could be effective as a potential anti-inflammatory drug in CF therapy.

53 Nanoscale characterisation of the effect of OligoG treatment on bacterial cell-surface charge and aggregation *in vitro*

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Pseudomonas aeruginosa (PA) is a common cause of morbidity in cystic fibrosis patients. Studies have demonstrated that OligoG, a defined alginate oligomer nanomedicine, potentiates the activity of conventional antibiotics by up to 500-fold against a range of multi-drug resistant Gram-negative bacteria. Using nanoscale analysis (surface charge and sizing measurements, and atomic force microscopy) interaction of OligoG with the surface of the PA strain PAO1 was characterised. Sizing analysis (employing the dynamic light scattering process) showed OligoG treatment resulted in a 2 fold increase in bacterial size (1500±220 nm), which was associated with cell-clumping. Hydrodynamic shear experiments demonstrated that this interaction was not displaced by centrifugation (5,500 *g*). Surface-charge distribution, characterised using zeta potential measurements, demonstrated the bimodal, heterogeneity of surface charge in PA (peak 1: −28.6±1.1 mV, peak2: −50.2±2.2 mV). OligoG treatment resulted in modulation of the bacterial surface charge with the development of a secondary, more negative zeta-potential peak (−57.8±2.7 mV). These results demonstrate that OligoG binds to the bacteria cell surface, causing it to become more negatively charged. The changes brought about by OligoG binding may influence microbial cell adhesion and hence biofilm formation. Further studies are ongoing to further elucidate these post-binding events.

54 Effects of the alginate oligosaccharide OligoG on the surface properties of Gram-negative bacterial biofilms using atomic force microscopy

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The importance of biofilms in chronic human disease is increasingly recognised in modulating the local immune response and reducing the efficiacy of conventional antimicrobial therapies. We have previously shown, using scanning electron and confocal laser scanning microscopy, that the clinically safe alginate oligomer OligoG disrupts *Pseudomonas aeruginosa* PAO1 biofilm formation. The ability of OligoG to affect established biofilms was quantified using atomic force microscopy (AFM) to map differences in the mechanical properties of biofilm surfaces. Dried biofilms were imaged in air using tapping mode operation with silicon nitride non-contact cantilevers. AFM imaging and force measurements demonstrated obvious differences in the surface mechanical characteristics of the OligoG-treated and untreated biofilms. OligoG-treated biofilms exhibited significantly lower Young's moduli, and hence were less mechanically robust than the untreated biofilm ($p < 0.05$). When treated and untreated biofilms were subject to a controlled hydrodynamic shear, the remaining biofilms had significant differences in surface roughness as measured from AFM images (35.2±7.6 nm vs 12.1±5.4 nm). These studies demonstrate the utility of AFM as a quantitative approach to investigate, at the nanoscale, the interaction of potential therapies on biofilm structures. They also show the potential ability for OligoG treatment to modulate biofilm structure and maintenance *in vivo*.